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Beyond Antiangiogenesis: Vascular Modulation as an Anticancer Therapy—A Review

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Abstract

This review attempts to move beyond the traditional borders of antiangiogenesis and toward the dynamic, evolving strategies of vascular modulation. This repositioning entails a two-fold paradigm shift: conceptually, to a view of antiangiogenesis as only one part of a larger story, and therapeutically, to approaches which attempt to modulate tumor blood flow instead of simply inhibiting it. Three vascular modulation strategies—provascular, antivascular, and redistributive—are presented with representative compounds. These vascular modulation strategies are described in specific measurable characteristics (blood vessel maturity and type, effect on blood flow, microenvironmental target, molecular target, angiogenic biomarker, and imaging biomarkers) that will help define the tumor types that are more susceptible to a particular vascular modulation strategy thereby guiding therapeutic agent selection and enabling a personalized medicine approach.

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Introduction

Cancer is a heterogeneous [1] and multifocal disease, not only between different individuals with neoplasms of the same histologic type but also often within the same tumor, resulting in unpredictable clinical outcomes. However, although no two cancers are identical [2], a universal feature of all solid tumors is their struggle to maintain blood flow to provide an adequate supply of oxygen and nutrients and to remove toxic metabolites as well as to serve as a pathway for metastasis. This dependence on a common pathway provides a potential therapeutic opportunity that replaces the tumor with a target of lesser complexity: unlike the cancer cells, the vascular endothelium is homogenous, genetically stable [3], and readily accessible through the bloodstream.

The normal adult vasculature is quiescent [4], with endothelial cells (ECs) dividing approximately every 10 years. In contrast, solid tumors require constant neovascularization to grow beyond ~1 mm^3 [5] in size; therefore, vascular modulation strategies to enhance, redirect, or limit tumor perfusion, provascular (fed), and redistributive and antivascular (fasted) strategies, respectively, represent promising anticancer approaches both as single agents and in combination with other modalities.

In effect, provascular and antivascular approaches can be considered as two sides of the same coin, exploiting the critical dependency of the tumor on blood flow as a vulnerability that can be manipulated—in either direction—to induce antitumor activity alone or in combination with other therapeutic modalities, such as chemotherapy or radiotherapy. Redistributive approaches, by contrast, represent an intermediate state that induces a *relative* change or reorganization of blood flow within the tumor through constriction or dilation of sections of the microvasculature, exploiting the heterogeneity of the tumor.

In general terms, from the perspective of a continuum (Figure 1), and for the convenience of classification, antivascular strategies limit blood flow and subject the tumor to hypoxia and nutrient stress at one end of the spectrum, whereas provascular strategies transiently improve the function of tumor blood vessels at the other. Redistributive approaches that result in intratumoral variation in blood flow without necessarily altering the tumor-to-normal tissue perfusion ratio represent an intermediate position encompassing both extremes. However, the position of these approaches on the continuum is very much dependent on the context, so an antivascular agent may become a provascular agent and *vice versa*. Cancer is a moving target, and the applicability and outcome of therapies that affect blood flow are similarly variable in a case-and-tumor-specific manner.

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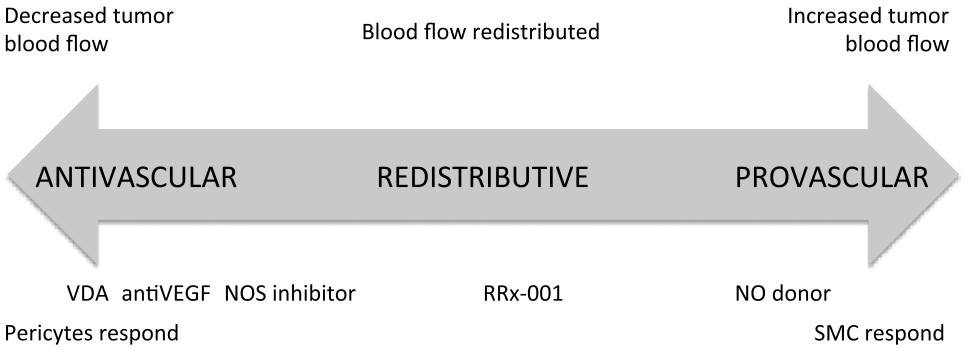

Figure 1. Vascular modulation continuum.

The field of tumor antiangiogenesis, built on the work of Judah Folkman [6], and although nascent, has rapidly generated a large body of multidisciplinary information, encompassing aspects of physics, mathematics, molecular biology, and engineering. However, rather than attempting exhaustively to explore and integrate the theoretical or conceptual underpinnings of the field, it is the aim of this review to extract key translational constituents and, using a top-down, "effect before cause" approach, synthesize them into six characteristics with potential clinical application (Table 1). The six characteristics are as follows:

1. Vessel maturity and type
2. Effect on blood flow
3. Microenvironmental target
4. Molecular target
5. Angiogenic biomarkers
6. Imaging biomarkers

These characteristics will be applied in turn to representative therapeutic classes of each of the three vascular modulation strategies. On the premise that conceptual clarity is inversely proportional to biologic complexity, we have focused on key interactions rather than incorporating a vast amount of information.

This review attempts to move beyond the traditional borders of antiangiogenesis and toward the dynamic, evolving strategies of vascular modulation. This repositioning entails a two-fold paradigm shift: conceptually, to a view of antiangiogenesis (and vascular disruption) as only one part of a larger story, and therapeutically, to approaches that attempt to modulate tumor blood flow instead of simply inhibiting it.

The unpredictable element, contributing to the variability of the response, is nitric oxide (NO), the reappearing multifacted regulator that, depending on the context, can influence the therapeutic outcome positively or negatively. Its pleiotropic role in vascular modulation and its important relationship to the smooth muscle containing pericyte (PC) that coats the ECs in the microvasculature will be emphasized throughout this review.

Antivascular Strategies

The old dictum "feed a cold, starve a fever" can be less succinctly restated in oncologic terms as "feed the normal tissue, starve a tumor." The requirements of this modified dictum are met by the vascular targeting agents, namely, the 1) the vascular disruptive agents (VDAs) that occlude preexisting vessels, 2) the anti-vascular endothelial growth factors (VEGFs) that prevent new blood vessel formation, 3) the NO synthase (NOS) inhibitors such as L-NNA and depending on the context, and 4) the vasodilators such as NO, which can reduce tumor perfusion through diversion of blood flow through the steal phenomenon [7].

Vascular Disruptive Agents

The vessel microenvironment. Fully functional blood vessels are composed of ECs and perivascular mural cells; for example, PCs and vascular smooth muscle cells (vSMCs) [8]. Without PCs, vessels are immature and require VEGF stimulation for continued survival. With PCs, ECs lose their dependence on VEGF, and blood vessels are characterized by a nonangiogenic, mature, stable phenotype [9]. Aggressive tumor angiogenesis is attributed to abnormal PC production of VEGF especially under hypoxia resulting in EC proliferation [10]. Essentially, the two cell types exhibit a marked functional interdependence and act as a structural unit to promote their mutual survival [11].

Table 1. Vascular Modifying Strategies.

	Strategy	Antivascular			Redistributive	Provascular
	Therapy Class	Vascular Disrupting Agents	Antiangiogenic Anti-VEGF Antibodies, VEGFR Kinase Inhibitors	NOS Inhibitors		NO Donors
	Compound Example	Combretastatin A-4	Bevacizumab (Avastin)	L-NNA	RRx-001	Nitroglycerin
1	Vessel maturity and type	Immature but established	Immature and newly formed	Mature	Mature	Mature
2	Effect on blood flow	Decreased	Decreased	Decreased	Redistributed	Increased/decreased (variable)
3	Microenvironmental target	ECs	ECs	Microvessels	Hemoglobin	PCs
4	Molecular target	Tubulin	Circulating VEGF	eNOS	NO	Guanylate cyclase
5	Angiogenic biomarker	α-SMA eNOS MVD	HTN, eNOS, α-SMA	eNOS, α-SMA	α-SMA, eNOS CD31, CD34	Blood pressure, α-SMA eNOS
6	Imaging modality	DCE-MRI	DCE-MRI	CEUS, DCE-MRI or DCE-CT	DCE-MRI, CEUS	DCE-MRI, CEUS

HTN indicates hypertension.

PCs are solitary vSMC-like cells [12] associated with the smallest diameter blood vessels, that is, arterioles, capillaries, and venules, whereas larger vessels such as arteries and veins are surrounded by concentric layers of vSMCs. Blood vessels initially form as endothelial tubes without PC contact. ECs secrete platelet-derived growth factor-BB leading to PC proliferation and migration [13] and the subsequent coverage of ECs by PCs results in vessels with a more mature and stable phenotype [14]. Conversely, tumor blood vessels are often characterized by a high microvessel density (MVD), a low ratio of PCs [15] to ECs, and/or an abnormally loose association [16], leading to vessel destabilization [17], abnormal morphogenesis [18], and impaired vasoregulation.

PCs possess contractile proteins [19] such as α smooth muscle actin (α-SMA), tropomyosin, and myosin. Similar to the smooth muscle cells of larger vessels, they can therefore regulate vascular diameter and capillary blood flow in response to vasoactive substances like prostacyclin, endothelin 1, and angiotensin II. NO, which is produced by ECs, mediates PC relaxation through guanylyl cyclase that is expressed in PCs [20]. Elevated levels of carbon dioxide can also induce relaxation, whereas hyperoxia increases PC contraction [21], thereby restricting blood flow. The presence and distribution of PCs in the tumor can be determined by immunohistochemical staining for α-SMA [22].

VDA: An overview of combretastatin A4P (Zybrestat). Combretastatin A4 phosphate (CA4P), the most advanced small-molecule VDA in clinical development and with some structural similarity to colchicine [23], binds the colchicine binding site on tubulin and disrupts the cytoskeleton of actively proliferating ECs [24] in PC-depleted tumor vessels, inhibiting their mitotic division and causing a reversible shutdown of tumor blood flow. However, mature PC-stabilized vessels are relatively resistant to CA4P [25]. The activity of the VDAs is typically confined to the hypoxic, VEGF-dependent vessels in the center of the tumor [26] while sparing the more mature and stable vasculature in the periphery. Recently, Zybrestat became available for compassionate use in patients being treated for anaplastic thyroid cancer in Canada and Europe. The vascular disrupting characteristics of Zybrestat are also being developed to target polypoidal choroidal vasculopathy.

Resistance to CA4P is also correlated with tumor overexpression of inducible NOS. Tozer et al. [27] and Davis et al. [28] have demonstrated enhanced efficacy of CA4P in previously unresponsive SaS tumors (increased NOS activity) in the presence of the NOS inhibitor, L-NNA, by suppression of the proangiogenic and vasodilatory effects of NO. Therefore, endothelial NOS (eNOS) expression in tumors, determined immunohistochemically, has a potential role as a surrogate predictor of activity in conjunction with α-SMA and VEGF. It is therefore possible that CA4P monotherapy demonstrated a significant survival benefit [29] in thyroid anaplastic carcinomas [30] that are characterized histologically by the presence of neovessels lacking PC support.

A phase 1 study of CA4P in combination with the anti-VEGF antibody, bevacizumab, showed statistically significant but reversible reductions in tumor perfusion/permeability by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) with CA4P alone that were sustained after bevacizumab [31]. A potential rationale for this observation is that addition of the anti-VEGF antibody resulted in devascularization of the residual tumor rim. In addition, the therapeutic enhancement could be attributed to the prevention of regrowth of the neovasculature and the inhibition of VEGF-induced release of NO from ECs.

Potential biomarker signatures predictive of CA4P therapeutic outcome and combination strategies. Tumor response to treatment is typically assessed by measuring tumor size with axial computed tomography (CT) or MRI, following the Response Evaluation Criteria in Solid Tumor guidelines. However, because combretastatin rarely results in immediate tumor shrinkage [32], anatomy-based imaging techniques may at best be a lagging indicator of the effectiveness of the VDA treatment regimen. T1-weighted DCE-MRI is a convenient modality that has been used in clinical trials with CA4P to track a bolus of Gd contrast agent for tumor perfusion measurements and vascular permeability characterization [33]. Monitoring tumor perfusion may be a way to predict tumor response early in the course and be used as a predictor of activity and be used as a guide to the optimal timing for the administration of combination therapy. A potential biomarker signature predictive of CA4P therapeutic outcome across multiple cancer types and subcategories of disease may be a low expression of eNOS, α-SMA, and VEGF, a high MVD, and a posttreatment reduction (within 24 hours) of tumor perfusion measured by DCE-MRI.

In addition to conventional chemotherapy, radiotherapy, and antiangiogenic therapy, VDAs could also be effectively combined with NO inhibitors and/or a hypoxia-selective agent like mitomycin C on the premise that the VDA will result in vessel shutdown and accentuate the hypoxia in the center of the tumor, thereby potentiating the activity of mitomycin C.

Antiangiogenic Agents: Bevacizumab (Avastin)

VEGF, bevacizumab, and the NO connection. A representative anti-VEGF inhibitor is a humanized monoclonal antibody (bevacizumab [Avastin]) approved by the US Food and Drug Administration as a first-line treatment for metastatic colorectal cancer in combination with 5-fluorouracil. The multitargeted tyrosine kinase inhibitors, sunitinib (Sutent) and sorafenib (Nexavar), which target VEGF in addition to other receptor tyrosine kinases, and the mTor inhibitors, temsirolimus (Torisel) and everolimus (Afinitor), which indirectly affect VEGF synthesis [34] are outside the scope of this review.

The VEGF family of growth factors consists in humans of five separate gene products, termed VEGF-A (or VEGF), VEGF-B, VEGF-C, VEGF-D, and placental growth factor, with roles in embryogenesis, development, and tissue remodeling. VEGF-A is generated in almost all cells, including ECs, under unfavorable stress conditions such as hypoxia and is a key driver of aberrant angiogenesis in tissues undergoing growth or remodeling [35], including cancers through binding to two tyrosine kinase receptors, VEGFR-1 (flt-1) and VEGFR-2 (flk-1/KDR), on the surface of ECs [36].

VEGF is secreted by PCs in the microvasculature especially under hypoxic conditions, establishing a paracrine loop of EC vasoproliferation [37]. Endothelial survival in newly formed vessels is VEGF-dependent and withdrawal of VEGF in vessels lacking PCs [38] results in selective apoptosis.

In addition, VEGF generates vasodilation and promotes increased vascular permeability [39], leading to the local extravasation of plasma proteins and matrix components that facilitate endothelial migration [40]. VEGF expression is regulated by several transcription factors, including hypoxia-inducible factor 1α, and mediators such as NO released by NOS.

VEGF-induced vasodilation and its effect on vascular permeability are dependent, at least in part, on the downstream effects of NO. The regulation is reciprocal: NO can upregulate VEGF by enhancing

hypoxia-inducible factor 1 activity [41]. The development of hypertension, occurring in 24% [42] of bevacizumab-treated patients, possibly due to the inhibition of NO synthesis, is associated with improved outcomes [43] and may serve as a correlate of efficacy.

Vascular normalization and VEGF inhibitors: Is bevacizumab antivascular or provascular?. Jain et al. [44] have coined the term *vascular normalization* to describe the pruning of immature or nascent vessels that lack a PC coating and the relative enrichment for PC-coated mature vessels. Immature vessels are hyperpermeable and tortuous; mature vessels are not. The prevention of neovasculogenesis and resultant decrease in interstitial pressure results in improved perfusion and oxygenation [45], paradoxically turning an antivascular agent into a provascular one.

However, in breast and colorectal studies, a consistently increased dose response was not observed in combination with standard chemotherapy (irinotecan/5-fluorouracil/leucovorin). In fact, treatment with 5 mg/kg bevacizumab seemed to be more effective than the 10-mg/kg dose in the colorectal study. This led at least one author [46] to speculate, somewhat paradoxically, that a relatively low-dose bevacizumab may have resulted in reduced intratumoral pressure and improved delivery of chemotherapy, whereas higher doses may have caused vascular collapse inside the tumor, thereby limiting delivery of chemotherapy and reducing the overall antitumor activity.

Biomarkers and imaging modalities. Bevacizumab has a clear biologic effect that depends on binding VEGF, thus blocking VEGF signaling. However, measurement of baseline levels of VEGF expression in metastatic colorectal, non–small cell lung, and renal cell cancers has failed to predict benefit, possibly because, although VEGF may be the most important proangiogenic factor in early-stage disease, in later-stage disease, compensatory responses come into effect, which exploit multiple pathways rendering the VEGF response less critical [47].

Hypertension may serve as a surrogate marker for anti-VEGF activity, but the association of bevacizumab with congestive heart failure [48] may exclude the treatment of patients with preexisting cardiac disease. In suitable patients, a high expression of eNOS in the tumor could be exploited as a surrogate biomarker of VEGF-dependent tumor growth because VEGF reciprocally upregulates NO.

DCE-MRI can be used to study changes in K_{trans}, a measure of permeability and perfusion. Significant lowering of this parameter after drug administration would reflect a decrease in tumor vascular permeability and/or flow and would be consistent with "vascular normalization."

A relative increase in the immunohistochemical staining intensity of α-SMA over time would also be consistent with vascular normalization.

Potential combination therapies. Acting as a provascular agent at low doses, bevacizumab has potential as a chemoradiosensitizer, increasing oxygen and drug delivery. Results of a small phase 1 to 2 trial in locally advanced rectal cancer with bevacizumab and erlotinib in combination with continuously infused 5-fluorouracil and radiotherapy demonstrated a pathologic complete response in 7 (47%) of 15 patients who completed study therapy, and no local recurrences were observed after a median follow-up of 7 months [49].

As an antivascular agent at high doses, bevacizumab could increase the degree and severity of hypoxia in tumors. Therefore, it would make sense to combine bevacizumab with preadministered hypoxia-activated agents like mitomycin C. In such a combination, the hypoxia-activated compound could exploit bevacizumab-induced increased hypoxic fraction to selectively kill cancer cells with decreased systemic toxicity.

NOS Inhibitors—L-NNA

eNOS, α-SMA, and NO. NO is generated endogenously by NOS in mammals from the oxidation of L-arginine to L-citrulline. NOS can be categorized into three isoforms: neuronal (nNOS or NOS-1; an "NO brainer"), cytokine-inducible (iNOS or NOS-2), and endothelial (eNOS or NOS-3) [50]. eNOS is regulated by multiple interdependent control mechanisms and signaling pathways [51] including VEGF. The activity of the enzyme directly promotes the relaxation of vSMCs and PCs through stimulation of NO generation, leading to a reduction in blood pressure.

The ability of NO generated by eNOS to modify tumoral hemodynamics depends on the presence of smooth muscle–containing PCs in the microvasculature. Vessels devoid of α-SMA are incapable of active vasoconstriction/vasodilation [52].

The close association between eNOS and smooth muscle provides an anatomic basis for reciprocal regulation in the presence of agonists/antagonists. eNOS has been reported to associate with globular actin, and this association increases the activity of the enzyme. In a study in cultured human umbilical vein ECs, eNOS agonists adenosine, histamine, salbutamol, and thrombin all caused an increase in association between eNOS and globular actin [53].

NO inhibition as an antivascular strategy. The inhibition of NO synthesis has tumor antivascular activity that can be attributed to the involvement of NO in tumor angiogenesis and the maintenance of vasodilator tone of tumor blood vessels. A clinical phase 1 dose escalation study demonstrated that a single intravenous dose of L-NNA, a nonspecific NOS inhibitor with slightly greater selectivity for eNOS, decreased vascular blood volume by 40%, as measured by dynamic contrast CT, in non–small cell lung tumors, an effect that is sustained 24 hours after treatment [54].

The disruption of the tumor vasculature was preceded by a mild increase in blood pressure that returned to baseline in 3 hours [55]. The investigators attributed this discrepancy between the duration of the antiangiogenic and the cardiovascular effects (24 *vs* 3 hours) to a differential dependence on NO in healthy tissue compared with cancerous tissue [55]. Unlike the cardiovascular system, which is subjected to tightly regulated homeostatic controls [55], the patency of vessels within these tumors is maintained by increased expression of NO. Therefore, the consequence of NO inhibition was a conversion of net vasodilation to frank vasoconstriction with a catastrophic collapse of blood flow.

Biomarkers and imaging modalities. Implicit in this assumption about the differential effect of NO is a diffuse expression of SMA in the tumor. The reversal from dilation to constriction implies the presence of PCs in the tumor endothelium, which can dilate or constrict in the presence or absence of NO. Tumors that express a high basal level of eNOS and α-SMA may be particularly susceptible to the effects of treatment with NO inhibitors. Doppler ultrasound with intravenously injected gas- or air-containing microbubbles (contrast-enhanced ultrasound or CEUS) could also be used as an imaging biomarker to quantify tissue perfusion. CEUS presents several advantages over other imaging techniques such as DCE-MRI and CT, including the use of microbubbles as blood pool agents, portability, availability, and absence of risk of nephrotoxicity and exposure to

radiation. Owing to their size, microbubbles remain strictly intravascular where they can be detected with high sensitivity and specificity.

Potential combination therapies. As an antivascular agent that could increase the degree and severity of hypoxia in tumors, these observations suggest some specific combination therapies. In a similar combination to high-dose bevacizumab with CA4P, L-NNA could be combined with preadministered hypoxia-activated agents like mitomycin C.

Systemic Vasodilators and Vasoconstrictors

Adjustments to systemic changes must take place at the local tumor level. The administration of a vasodilator or a vasoconstrictor will result in decreased tumor perfusion if PCs that can respond to vasoactive stimuli are largely absent and the host and vascular beds are in parallel rather than in series [56]. However, because of the nonhomogenous distribution of vascular beds in most tumors, the net response to changes in systemic blood pressure is highly unpredictable and heterogeneous.

For example, the use of vasoconstrictors such as endothelin and angiotensin II [57] can lead to either improved blood flow to the tumor from unequal vasoconstriction of host vessels or decreased tumor blood flow, if the tumor endothelium expresses receptors that mediate vasoconstriction.

Similarly, vasodilators like NO donors can either mediate increased or decreased tumor perfusion, secondary to microvessel relaxation or to vascular steal, an effect that is due to a greater reduction of the total resistance of the peripheral circulation diverting blood away from the tumor. NO-based therapies, in particular, will be discussed in greater detail in the next paragraphs under provascular strategies.

Redistributive Strategies

Provascular and antivascular strategies involve a general or global change in perfusion to the tumor. Redistributive strategies, by contrast, induce local hemodynamic variations at the microregional level of the tumor, shifting blood flow and oxygenation into reconfigured zones, without necessarily influencing global perfusion. This hemodynamic reapportionment has the potential to minimize systemic effects and thereby improve the therapeutic index both as monotherapy and in combination because perfusion in the rest of the organism is differentially less affected.

By this definition, the administration of L-NNA to patients with non–small cell lung carcinoma represents a hybrid of antivascular and redistributive strategies because a global decrease in tumor perfusion (antivascular) was achieved with minimal effects on systemic hemodynamics (redistributive).

A key factor governing the sensitivity of the vasculature to therapeutic intervention is the abundance and distribution of α-SMA. Given the phenotypic heterogeneity of cancer, the effects of L-NNA could vary in different tumor types and even in individual patients. If the distribution of PCs in the tumor is focal, then the overall effect of L-NNA–induced NO inhibition would be antivascular through vasoconstriction in that zone. If the PCs are diffusely present in the tumor, then the net effect might be redistribution through siphoning of blood from PC-constricted microvessels to adjacent nonconstricted microvessels without PCs. Overall, blood flow and oxygenation are gerrymandered from one area of the tumor to another, but quantitative tumor perfusion may remain relatively stable.

The immature, unstable vessels in the tumor lacking PCs are prone to acute or transient hypoxia, resulting from intermittent closure. A redistributive agent that redirects blood flow in this way could lead to changes in the acute hypoxic pattern owing to the opening and closure of different blood vessels.

Redistribution is a novel and unique strategy. The ability to test this hypothesis has been hampered by the lack of chemical agents; however, we now have access to such a molecule in RRx-001.

RRx-001

RRx-001, an example of a small molecule parenterally dosed redistributive agent, is in phase 1 clinical evaluation in patients with advanced solid tumors and lymphomas [58]. The compound, sourced from the defense industry, and modified from a component in rocket fuel, is characterized by a pharmacologically unprecedented chemical structure, a pernitro-substituted strained four-member ring and possesses a novel mechanism of action. The molecule consists of two functional ends: a highly electrophilic center that rapidly reacts with hemoglobin and specific soluble thiols, depleting glutathione and a latent NO-donating moiety that is activated subsequent to reduction of the nitro groups on the four-member ring [59]. The covalent modification of hemoglobin through binding of RRx-001 to the β-Cys93 residue changes the oxygen affinity (lowering P50) and increases the capacity of the red blood cells to generate NO from nitrite (NO_2^-) [60].

Paradoxically, for a putative NO donor, RRx-001 administration in dogs led to a transient and mild increase in blood pressure, whereas mouse SCCVII and RIF-1 syngeneic tumors responded with a diffuse sustained vasoconstriction that resulted in blood flow redistribution for up to 72 hours and significant tumor cytotoxicity with minimal systemic adverse effects at therapeutic doses, both as monotherapy and in combination with radiotherapy (XRT).

The rationale for this perfusion redistribution is that murine squamous cell carcinoma cell line (SCCVII) and murine radiation-induced fibrosarcoma cell line (RIF-1) syngeneic murine tumors stain diffusely for α-SMA vessels; RRx-001 led to an acute change in blood flow and hypoxia distribution owing to the opening of immature (PC-poor) vessels and the closure of mature (PC-rich) vessels (RadioRx, unpublished observations, 2012). In the context of a glioma xenograft, U87, where the distribution of α-SMA was sparsely focal, RRx-001 had an antivascular, rather than a redistributive, effect, resulting in transient vasoconstriction and increased hypoxia, as assessed by staining with the oxygen dependent probe, pimonidazole. In effect, RRx-001, as a mixed NO inhibitor, dispenses NO in to the circulation but results in rapid NO indirect scavenging also through superoxide anion generation from glutathione depletion. The biocharacter of RRx-001 can be shifted from single-agent NO antagonist to NO superagonist when combined with nitrite, which undergoes an accelerated catalytic reduction to NO by RRx-001–modified hemoglobin.

A treatment regimen alternating between single-agent RRx-001 and a combination of RRx-001 with sodium nitrite or XRT, to stress the tumor with cyclical fluctuations in perfusion and reactive nitrogen species may prevent tumor adaptation and escape mechanisms. This approach may be especially useful when combined with XRT in particular owing to dynamic changes in tumor oxygenation and nitrosation that will enhance radiosensitization. To support this hypothesis, a biomarker "portfolio" predictive of activity will be evaluated in the clinic and includes eNOS, α-SMA, CD31, CD34, thrombospondin-1, DCE-MRI, and CEUS. On the basis of observed

preclinical radiosensitization, a phase 2 study is planned with XRT in selected patients with susceptible tumor types based on the analysis of this biomarker portfolio in phase 1.

Provascular Strategy—Nitric Oxide

The term *provascular*, introduced by Sonveaux et al. [61], refers collectively to agents that exploit the differential reactivity of mature tumor blood vessels to increase tumor perfusion and oxygenation temporarily. If the benefits of antiangiogenesis can be understood simplistically in oxygen deficiency and nutrient deprivation to the tumor leading to cytostasis, the advantages of a provascular strategy from improved oxygenation and drug delivery are less apparent and seemingly outweighed by potential stimulation of tumor growth associated with increased perfusion.

Pharmacokinetic modulation to increase tumor blood flow has been investigated with several agents including NO [62]. The utility of NO as a provascular agent depends on the functional role of PCs in the tumor vasculature; transient relaxation of these cells may temporarily increase blood flow. Conversely, however, because normal tissue blood vessels are highly sensitive to vasodilation, and tumor perfusion pressures are influenced by systemic perfusion pressures, a reduction in tumor blood supply can also occur through vascular steal. Therefore, selective tumor vascular reactivity is dependent on local delivery of NO to the tumor from, for example, local low-dose radiation, which induces a dose-dependent up-regulation of eNOS [63] or an NO donor like RRx-001, which functionalizes hemoglobin and, in combination with nitrite, converts red blood cells into circulating bioreactors that preferentially deliver NO on-demand under hypoxia.

However, although it is extremely attractive to "starve" a tumor into nongrowth or growth at submaximal rates in principle, antiangiogenesis has been disappointing in practice. Because hypoxia is the major driver of malignant progression, angiogenesis, and treatment resistance, the benefits to the patient and the clinician of tumor reoxygenation and a reduction in the hypoxic fraction may actually favor a provascular rather than an antivascular approach. The provascular paradox is that normalization of the tumor vasculature can lead to improved blood flow, making the distinction between provascular and antivascular agents somewhat artificial because both strategies are contextually morphable depending on the dose and the tumor type. According to Sonveaux et al., the real benefit of a selective provascular approach is that, because tumor tissues are more hypoxic and less perfused than normal tissues, the impact on the tumor of even a small enhancement of oxygenation and blood flow is substantial, resulting in chemoradiosensitization.

In addition, when the tumor vasculature is PC-poor, systemic delivery of a vasoconstrictor, such as angiotensin II, rather than a vasodilator, will indirectly increase blood flow to the tumor, through an anti–steal effect involving vasoconstriction of normal, but not tumor, blood vessels. Important systemic and biopsy biomarkers could include blood pressure (because a precipitous decrease would herald a decrease in tumor perfusion through steal) and eNOS and α-SMA, respectively.

Conclusions

It has often been stated that cancer is not one disease but more than a hundred different diseases that present variable clinical courses and treatment challenges [64]. The attraction of vascular modulation is that it addresses the therapeutic disadvantages of cancer heterogeneity: the common denominator of all tumors regardless of histologic features, biologic profile, and imaging characteristics is a complete reliance on blood flow to maintain growth, progression, and metastases. This reliance is the Achilles' heel of cancer, and the ability to manipulate and exploit it using vasoactive and antivascular agents, both separately and in combination, as a means of destroying the tumor itself, is the Holy Grail in oncology.

Although deceptively simple, the tumor vasculature is no different from the tumor in its heterogeneity, and the kaleidoscopic interplay between the components of the microenvironment—the ECs, the smooth muscle—containing PCs and endogenous angiogenic factors, such as VEGF and NO, which influence the treatment outcome. From an understanding of the complexities of the tumor, vasculature and microenvironment have emerged a continuum of strategies, from antivascular to provascular, to target blood flow as a whole and not just the epithelial component of the neovasculature. Moreover, the absence of a single therapeutic target for the tumor vasculature has led to an emphasis on rational combinations of therapies chosen on the basis of particular "biomarker signatures" predictive of microenvironmental conduciveness in individual tumors.

The continuum presented in the introduction is, of course, an oversimplified construct, but it provides a conceptual taxonomy for the different strategies in this review. None of the strategies are intentionally presented as "better" or more "desirable" than another. In fact, depending on the context, they are relatively interchangeable. The continuum is a static representation, and the therapies that are identified as belonging to discrete categories—antivascular, redistributive, or provascular—are capable of dynamically switching their effect on blood flow, mirroring the adaptive response of the tumor itself to vasoactive challenges. This therapeutic fickleness can be manipulated to fine-tune the vascular response through dose adjustment or addition of a second modality.

The mismatch between static, linear treatment regimens with fixed maximum tolerated doses (MTDs) and schedules and the dynamic evolutive responses of tumor cells to chemotherapy and radiotherapy in some cancers inevitably results in the development of therapeutic resistance. Moreover, treatment failure is typically determined *post hoc* with static imaging instead of *ad hoc* with dynamic MRI or CEUS, exacerbating the disconnect.

In contrast to this conventional Response Evaluation Criteria in Solid Tumor and MTD-based clinical methodology, vascular modulation strategies have the potential to provide a flexible platform for iterative therapeutic tailoring based on rapid feedback: treatment can be continuously adapted to real-time changes in tumor blood flow with dynamic CEUS and/or DCE-MRI.

In this therapeutic serve-and-volley between provascular and antivascular approaches, the clinician theoretically has the advantage because, with every phenotypic adaptation, the tumor pays an efficiency price, diverting energy and resources from invasion to evasion, thereby rendering it more vulnerable to subsequent therapeutic attacks.

Thus, in conclusion, the prospect of vascular modulation strategies that, through biomarker identification and serial imaging techniques, can become as adaptive and dynamic as the tumor itself offers new hope and new opportunities for improving the care of cancer patients.

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